

The Role of Aloe vera gel and Butyrate Fermented to Cell Apoptosis and Angiogenesis; Case Reports: Wound Healing of a Scar of the Scald and Hair Growth

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ABSTRACT

We discussed the efficacy of butyrate fermented in aloe vera gel for the regulation of inflammation, carcinogenesis, and cell apoptosis and angiogenesis. Additionally we reported a potential bioactivity of aloe vera gel for wound healing and hair growth in case reports.

Key words: Butyrate fermented; Prevention of angiogenesis; Case reports

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INTRODUCTION

Aloe vera has been used as a traditional medicine for inducing wound healing. Acemannan, one of a polysaccharide in Aloe vera extracts containing pectin, glycoprotein, and protein stimulates cell proliferation and skin wound healing through AKT/mTOR signaling pathway mediated protein translation mechanism, which may notify an alternative therapy approach for cutaneous wound^[1]. Apoptosis is a natural process that is controlled by genetically in normal cell development and regulated by expression of many genes included mitogens and their receptor, RAS/MAPK and nuclear factor kappa B cells (NF- κ B). In the presence of IFN- γ acemannan induced apoptosis in RAW 264.7 cells. These cells exhibited chromatin condensation, DNA fragmentation, and laddering characteristic of apoptosis. The evidence suggested that aloe vera extracts can induce apoptosis and lymphocyte function^[2]. Aloe polysaccharide (AP) was shown that AP has radioactive effects on normal human cells in vitro and mouse survival in vivo. AP could lower thymocyte apoptosis of mice in vivo after 6 Gy-irradiation and abrogate the cell cycle perturbation. AP exerted radio-protective effects in vitro and in vivo through an inhibition of apoptosis^[3]. Brain tumor, glioblastoma, is one of most serious malignant tumor of central nervous system. The role of aloe vera extract was investigated in regulation of apoptosis through p38 and NF- κ B gene against human glioma U87MG cells in vitro^[4]. Flavonoid (or aloe-emodin) showed inhibitory effect on glioblastoma, a brain tumor cells, by apoptosis through regulation of p38 and NF- κ B pathway at transcription and post-transcription level.

Aloe vera has long been used as a traditional medicine for inducing wound healing. Gingival fibroblasts (GFs) play important roles in the proliferative phase of oral wound healing. They proliferate and secrete several growth factors and extracellular matrix, such as keratinocyte growth factor-1(KGF-1), vascular endothelial growth factor (VEGF), and type I collagen production, and oral wound healing in rats. Wound healing of animal receiving Carbopol

containing 0.5% acemannan was significantly better than that of the other groups. The findings suggested that acemannan plays a significant role in the oral wound healing process via the induction of fibroblast proliferation and stimulation of KGF-1, VEGF, and type I collagen expression^[5]. Although the beneficial effects of aloe vera on wound healing have been proven, little is unknown about the effects on the cellular level. Shafaie *et al*^[6] appraised the angiogenic and migratory effects of aloe vera gel on fibroblasts and endothelial cells. Aloe vera gel induced angiogenic and cell adhesion properties in fibroblasts rather than endothelial cells in wound healing by aloe vera administration.

In the present review, we explore possible prophylactic contributions of aloe vera gel, butyrate fermented to pro-apoptosis and anti-angiogenesis on human cancer cells. In case reports we described wound healing of a scar of the scald and hair growth with aloe vera juice ingestion.

ANGIOGENESIS IN CANCER

New blood and lymphatic vessels form through processes called angiogenesis and lymph-angiogenesis, respectively. Angiogenesis is regulated by a balance between activators and inhibitors. More than a dozen proteins have been identified as angiogenic activators and inhibitors. Levels of expression of angiogenic factors mirror the aggressiveness of tumor cells. The discovery of angiogenic inhibitors should help to reduce both morbidity and mortality from carcinomas^[7].

THE EFFECT OF BUTYRATE ON THE GROWTH OF EHRlich ASCITES TUMOR CELLS IN VIVO

A short chain fatty acid, butyrate induces cell cycle arrest, apoptosis in transformed cell lines. Butyrate on the growth of Ehrlich ascites tumor (EAT) cells *in vivo* was investigated^[8]. Butyrate, when injected intraperitoneally into mice inhibited proliferation of EAT cells. And induction of apoptosis in EAT cells was monitored by nuclear condensation, annexin-V staining, DNA fragmentation, and translocation of caspase-activated DNase into nucleus upon butyrate-treatment. The pro-apoptotic effect of butyrate reflects on the antiangiogenic pathway in EAT cells. The results suggest that butyrate, besides regulating other fundamental cellular processes, is able to modulate the expression/secretion of the key angiogenic growth factor VEGF in EAT cells. In previous paper we showed that the probiotic/prebiotic strategies can modulate an endogenous HDAC inhibitor, butyrate, for anti-cancer chemoprevention without the adverse effects^[9].

THE ANTI-ANGIOGENESIS ROLE OF HISTONE DEACETYLASE INHIBITORS

Angiogenesis, a process of new blood vessel formation from the existing blood vessels, plays an important role in tumor growth and the tissue repair process. In tumor growth, angiogenesis often increases oncogenic signaling, and in tissue repair, it decreases the stiffness of wound tissue and potentially exacerbates scar formation, resulting in pain and poor function. Most studies have indicated that HDAC inhibitors show great promise as antiangiogenic agents in the early phase of clinical trials. The first HDAC inhibitor approved by the FDA for cancer therapy is suberoylanilide hydroxamic acid for treatment of cutaneous T-cell lymphoma. Deng *et al* discussed

the molecular and cellular underpinnings of the effects of HDAC inhibitors on angiogenesis^[10]. Angiogenesis is a critical factor in the development of tumors and metastases in numerous cancers. An increased level of angiogenesis is associated with decreased survival in breast cancer patients. Therefore, a good understanding of the angiogenesis mechanism hold a promise of providing effective treatments for breast cancer progression, thereby enhancing patient's survival. Madu *et al*^[11] reviewed the mechanism of angiogenesis in diseases, specially its role in the progression of malignancy in breast cancer, as well as to highlight the undergoing research in the development of angiogenesis-targeting therapies.

THE FUNCTIONS OF G-PROTEIN-COUPLED RECEPTORS, GPR109A AND GPR43, FOR THE BACTERIAL FERMENTED BUTYRATE

GPR109A is a G-protein-coupled receptor or nicotinate but recognizes butyrate with low affinity. Milli-molar concentrations of butyrate are needed to activate the receptor. Thangaraju *et al* showed that GPR109A is expressed in the lumen-facing apical membrane of colonic and intestinal epithelial cells and indicated that the receptor recognizes butyrate as a ligand^[12]. Butyrate is an inhibitor of histone deacetylases, but apoptosis induced by activation of GPR109A with its ligands in colon cancer cells does not involve inhibition of histone deacetylation. If GPR109A is expressed in the lumen-facing apical membrane of colonocytes, it might be suggest that the ability of butyrate to prevent cancer and inflammation in the colon may be mediated extracellularly via the receptor without entering into cells.

Liu *et al*^[13] demonstrated that low concentrations of butyrate may contribute to healing of tendon-bone injury in part at least through promotion of tissue remodeling. The authors investigated the effects of low-range concentrations of sodium butyrate by use of *in vitro* and *in vivo* angiogenesis assay, as the primary outcome measure and mechanisms through which it acts. Sodium butyrate (SB) has been shown to stimulate angiogenesis at low doses in a vascular repair model *in vivo* and any detected proangiogenic changes establish the potential of SB to stimulate repair in a wound healing at a site where vasculature is low and healing is compromised. Furthermore the authors investigated the contribution of the SCFA receptor GPR43 in the proangiogenic effects of local treatment with SB and its effects on matrix remodeling using the sponge-induced fibro-vascular tissue model in mice lacking the GPR43 gene and the wild-type mice^[14]. The findings evidence that the metabolite-sensing receptor GPR43 contributes to the effects of low dose of butyrate in inducing angiogenesis and matrix remodeling during granulation tissue formation in mice.

THE ROLES OF BUTYRATE FOR MICRNAs, APOPTOSIS, AND AUTOPHAGY IN CANCER THERAPY

MicroRNAs (mRNAs) are a class of small non-coding RNAs, 20-22 nucleotides in length, located at intragenic and intergenic of the genome. The regulation of mRNA expression in the gut intestine is gradually recognized as one of the crucial contributors of intestinal homeostasis and overall health. Both the mRNAs endogenous in the gut intestine and exogenous from diets play influential roles in modulating microbial colonization and intestinal immunity. Bi *et al*^[15] discussed the biological functions of mRNAs in regulating intestinal homeostasis by modulating intestinal immune responses

and gut microbiota. mRNAs play a powerful and essential role in post-transcriptional gene regulation, which contributes to cell differentiation, proliferation, and apoptosis.

Both mRNA and autophagy, separately or concurrently, are involved in a variety of the cellular and molecular processes of multiple cancers; including urologic, malignancies. Shen *et al*^[16] discussed that mRNAs-mediated autophagy acted as carcinogenic factors or suppressors in prostate cancer, kidney cancer, and bladder cancer.

In the previous paper^[17] we showed that the importance of butyrate fermented in endophytic bacteria in aloe vera gel and host-mRNAs-microbiota interaction in regulation of gut immunity, and butyrate is a chemical factor capable of facilitating induced pluripotent stem cell generation, an important step for the establishment of pluripotency.

THE ROLE OF BUTYRATE INHIBITING TUMOR PROGRESSION VIA SUPPRESSING HISTONE DEACETYLASE

Butyrate inducing reactive oxygen species-mediated apoptosis

The role of miR-22 in butyrate-mediated reactive oxygen species (ROS) release and induction of apoptosis was concluded in hepatic cells by Pant *et al*^[18]. Butyrate induced apoptosis via ROS production, cytochrome c release and activation of caspase-3, whereas addition of N-acetyl cysteine (NAC) or anti-miR-22 reversed these butyrate-induced effects. When ROS was neutralized using NAC neither butyrate nor miR-22 could induce apoptosis in hepatic cells, confirming that butyrate induced miR-22, which in turn down-regulated Sirt-1 expression and enhanced ROS release.

Butyrate inhibiting colorectal cancer cell migration

The miR-200 family is a key regulator of the epithelial-mesenchymal transition (EMT). Xu *et al*^[19] investigated the role of miR-200s expression on cell migration in sodium butyrate (SB)-treated colorectal cancer cell (CRC). SB reciprocally increased miR-200s, but reduced expression of their target genes (Bmi-1). Upregulation of Bmi-1 expression partially reversed the effect of SB. SB inhibits CRC cell migration by enhancing miRNA-200s expression-mediated downregulation of Bmi-1. The findings support the utility of SB in colorectal cancer therapy.

Butyrate inhibiting migration and inducing AMPK-mTOR pathway-dependent autophagy and reactive oxygen species-mediated apoptosis

Wang *et al*^[20] estimated the anticancer effects, including cell migration inhibition and the apoptosis effects of butyrate in human bladder cancer cells. The authors found that butyrate inhibited migration and induced AMPK/mTOR (AMP-activated protein kinase/Toll-like receptor) pathway-activated autophagy and reactive oxygen species (ROS) overproduction via the miR-135-5p/Bmi-1 axis. Furthermore, the author determined ROS overproduction contributed butyrate-induced caspase-dependent apoptosis and autophagy. The interplay between autophagy and apoptosis in butyrate treatment was clearly refined.

CASE REPORT 1

Treatment of aloe vera to wound healing of a scar of the scald

A 67-years-old frail female who had liver trouble and aloe vera gel successive ingestion, had a scald in left hand in kitchen work on July, 2006 (Figure 1). A cooling with water at once and spreading with fresh aloe vera gel during three days, she had been a well QOL (Figure 2).

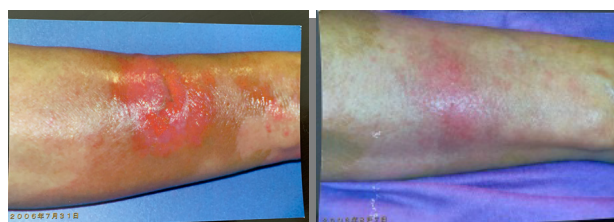


Figure 1 The scald in left hand. Figure 2 The spread left hand with aloe vera gel during three days.



Figure 3-8 The hair growth of the patient with hypertension started on September 21, 2020 and completed on September 5, 2021 with aloe vera juice ingestion.

CASE REPORT 2

Effect of aloe vera juice ingestion to hypertension and hair growth stimulation

A 51-years-old male having high uric acid level and hypertension (the diastolic blood pressure; around 200mm Hg), administered amlodipine and olmesartan with aloe vera juice (AVJ) 250 ml on September, 2020. He had a little of head swing but decided to drug deprivation and only take AVJ on October, 2020. Then he had blood examination; uric acid level, 6.5 mg/dL and diastolic blood pressure 166 and systolic blood pressure 106 mm Hg on January, 2021. Since then he has been well condition and surprisingly, his hair gradually grow and hair growth was completed with AVJ ingestion on September, 2021. He has been well QOL (Figures 3-8).

DISCUSSION IN CASE REPORT

Aloe vera gel is a powerful healer that has been successfully employed for millennia. It acts in the manner of conductor, orchestrating many biological active ingredients such as hyaluronan^[21] with polysaccharide fraction and fermented short chain fatty acids (SCFAs), such as butyric acid^[22], to achieve wound healing in frail 67-years-old-woman having liver trouble in Case report 1.

A 51-years-old male having high uric acid level and hypertension decided drug deprivation and took only successive AVJ ingestion for several months. Then he completed remission of uric acid level and hypertension. And hair growth was recovered in Case report 2. As shown in case report 2, the beneficial role of aloe vera gel to improve uremic toxins and uric acid was reported in our previous report^[23]. Stimulation of hair growth in 51-years old male was shown by ingestion of aloe vera juice.

SUMMARY

Two major SCFAs signaling mechanisms are promotion of histone acetylation and activation of G-protein-coupled receptors. We discussed the efficacy of SCFAs; regulation of inflammation, carcinogenesis, and cell apoptosis and angiogenesis by use of aloe vera gel. Possible prophylactic roles of butyrate fermented in aloe vera gel on kidney function and chronic hepatitis were discussed. In case reports we showed a potential bioactivity of successive ingestion of aloe vera juice for wound healing and the remission of hypertension and high level of uric acid. The daily ingestion of aloe vera juice may support hair growth.

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